

In the Specification:

Please replace the paragraph after the title with the following paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Application No. 08/874,109, filed June 12, 1997, which is a continuation-in-part of PCT Application No. PCT/US96/20090, filed December 13, 1996, which is a continuation-in-part of U.S. Application No. 08/572,027, filed December 14, 1995, all of which are incorporated by reference in their entirety.

Please amend the paragraph beginning at page 1, line 19 as follows:

Many breeding studies have been conducted to improve the fatty acid profile of *Brassica* varieties. Pleines and Freidt, *Fat Sci. Technol.* 90(5), 167-171 (1988) describe plant lines with reduced C_{18:3} levels (2.5-5.8%) combined with high oleic contents (73-79%). Rakow and McGregor, *J. Amer. Oil Chem. Soc.*, 50, 400-403 (Oct. 1973) discuss problems associated with selecting mutants for linoleic and linolenic acids. [[In.]] In *Can. J. Plant Sci.*, 68, 509-511 (Apr. 1988) Stellar summer rape producing seed oil with 3% linolenic acid and 28% linoleic acid is disclosed. Roy and Tarr, *Z. Pflanzenzuchtg*, 95(3), 201-209 (1985) teaches transfer of genes through an interspecific cross from *Brassica juncea* into *Brassica napus* resulting in a reconstituted line combining high linoleic with low linolenic acid content. Roy and Tarr, *Plant Breeding*, 98, 89-96 (1987) discuss prospects for development of *B. napus* L. having improved linolenic and linolenic acid content. European Patent application 323,753 published July 12, 1989 discloses seeds and oils having greater than 79% oleic acid combined with less than 3.5% linolenic acid. Canvin, *Can. J. Botany*, 43, 63-69 (1965) discusses the effect of temperature on the fatty acid composition of oils from several seed crops including rapeseed.

Please amend the paragraph beginning at page 6, line 4 as follows:

Figure 2 shows the nucleotide sequences for a *Brassica* Fad2-D wild type gene (Fad2-D wt; SEQ ID NO:9), IMC129 mutant gene (Fad2-D GA316 IMC129; SEQ ID NO:11), Fad2-F wild type gene (Fad2-F wt; SEQ ID NO:13), Q508 mutant gene (Fad2-F TA515 Q508; SEQ ID NO:15) and Q4275 mutant gene (Fad2-F GA908 Q4275; SEQ ID NO:17).

Please amend the paragraph beginning at page 6, line 9 as follows:

Figure 3 shows the deduced amino acid sequences (SEQ ID NOS:10, 12, 14, 16, and 18) for the polynucleotides of Figure 2.

Please amend the paragraph beginning at page 8, line 3 as follows:

Applicants have further discovered isolated nucleic acid fragments (polynucleotides) comprising sequences that carry mutations within the coding sequence of delta-12 or delta-15 fatty acid desaturases. The mutations confer desirable alterations in fatty acid levels in the seed oil of plants carrying such mutations. Delta-12 fatty acid desaturase is also known as omega-6 fatty acid desaturase and is sometimes referred to herein as Fad2 or 12-DES. Delta-15 fatty acid desaturase is also known ~~[[on]]~~ as omega-3 fatty acid desaturase and is sometimes referred to herein as Fad3 or 15-DES.

Please amend the paragraph beginning at page 10, line 29 as follows:

Preferred mutations are in a region of the nucleic acid encoding an amino acid sequence motif that is conserved among delta-12 fatty acid desaturases or delta-15 fatty acid desaturases, such as a His-Xaa-Xaa-Xaa-His motif (Tables 1-3). An example of a suitable region has a conserved HECGH motif (SEQ ID NO:60) that is found, for example, in nucleotides corresponding to amino acids 105 to 109 of the *Arabidopsis* and *Brassica* delta-12 desaturase sequences, in nucleotides corresponding to amino acids 101 to 105 of the soybean delta-12 desaturase sequence and in nucleotides corresponding to amino acids 111 to 115 of the maize delta-12 desaturase sequence. See e.g., ~~[[WO 94/115116]]~~ WO 94/11516; Okuley et al., Plant Cell 6:147-158 (1994). The one letter amino acid designations used herein are described in Alberts, B. et al., Molecular Biology of the Cell, 3rd edition, Garland Publishing, New York, 1994. Amino acids flanking this motif are also highly conserved among delta-12 and delta-15 desaturases and are also suitable candidates for mutations in fragments of the invention.

Please amend the paragraph beginning at page 11, line 15 as follows:

An illustrative embodiment of a mutation in a nucleic acid fragment of the invention is a Glu to Lys substitution in the HECGH (SEQ ID NO:60) motif of a *Brassica* microsomal delta-12

desaturase sequence, either the D form or the F form. This mutation results in the sequence HECGH (SEQ ID NO:60) being changed to HKCGH (SEQ ID NO:58) as seen by comparing amino acids 105-109 of SEQ ID NO:10 (wild-type D form) to amino acids 105-109 of SEQ ID NO:12 (mutant D form). A similar mutation in other Fad-2 sequences is contemplated to result in a non-functional gene product. (Compare SEQ ID NO:2 to SEQ ID NO:4).

Please amend the paragraph beginning at page 11, line 32 as follows:

Among the types of mutations in an HECGH (SEQ ID NO:60) motif that render the resulting gene product non-functional are non-conservative substitutions. An illustrative example of a non-conservative substitution is substitution of a glycine residue for either the first or second histidine. Such a substitution replaces a charged residue (histidine) with a non-polar residue (glycine). Another type of mutation that renders the resulting gene product non-functional is an insertion mutation, e.g., insertion of a glycine between the cysteine and glutamic acid residues in the HECGH (SEQ ID NO:60) motif.

Please amend the paragraph beginning at page 12, line 9 as follows:

Other regions having suitable conserved amino acid motifs include the HRRHH motif (SEQ ID NO:61) shown in Table 2, the HRTHH motif (SEQ ID NO:62) shown in Table 6 and the HVAHH motif (SEQ ID NO:63) shown in Table 3. See, e.g., [[WO 94/115116]] WO 94/11516; Hitz, W. et al., *Plant Physiol.*, 105:635-641 (1994); Okuley, J., et al., *supra*; and Yadav, N. et al., *supra*. An illustrative example of a mutation in the region shown in Table 3 is a mutation at nucleotides corresponding to the codon for glycine (amino acid 303 of *B. napus*). A non-conservative Gly to Glu substitution results in the amino acid sequence DRDYGILNKV (SEQ ID NO:47, amino acids 299-308 of SEQ ID NO:14) being changed to sequence DRDYEILNKV (SEQ ID NO:50, amino acids 299-308 of SEQ ID NO:18) (compare wild-type F form SEQ ID NO:14 to mutant Q4275 SEQ ID NO:18, Fig. 3).

Please amend the paragraph beginning at page 12, line 21 as follows:

Another region suitable for a mutation in a delta-12 desaturase sequence contains the motif KYLNNP (SEQ ID NO:64) at nucleotides corresponding to amino acids [[171]] 170 to 175

of the *Brassica* desaturase sequence. An illustrative example of a mutation [[is]] in this region is a Leu to His substitution, resulting in the amino acid sequence (Table 4) KYHNNP (SEQ ID NO:53, compare wild-type Fad2-F amino acids 170-175 of SEQ ID NO:14 to mutant Fad2-F amino acids 170-175 of SEQ ID NO:16). A similar mutation in other Fad-2 amino acid sequences is contemplated to result in a non-functional gene product. (Compare SEQ ID NO:6 to SEQ ID NO:8).

Please amend Table 1 on page 13 as follows:

TABLE 1

Alignment of Amino Acid Sequences from Microsomal
Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
<i>Arabidopsis thaliana</i>	100-129	IWVIAHECGH HAFSDYQWLD DTVGLIFHSF (SEQ ID NO:27)
<i>Glycine max</i>	96-125	VWVIAHECGH HAFSKYQWVD DVVGLTLHST (SEQ ID NO:28)
<i>Zea mays</i>	106-135	VWVIAHECGH HAFSDYSLLD DVVGLVLHSS (SEQ ID NO:29)
<i>Ricinus communis</i> ^a	1- 29	WVMAHDCGH HAFSDYQLLD DVVGLILHSC (SEQ ID NO:30)
<i>Brassica napus D</i>	100-128 ^b	VWVIAHECGH HAFSDYQWLD DTVGLIFHS (SEQ ID NO:65)
<i>Brassica napus F</i>	100-128 ^c	VWVIAHECGH HAFSDYQWLD DTVGLIFHS (SEQ ID NO:65)

^a from plasmid pRF2-1C; ^bpositions 100-128 of SEQ ID NO:10; ^cpositions 100-128 of SEQ ID NO:14

Please amend Table 2 on page 13 as follows:

TABLE 2

Alignment of Amino Acid Sequences from Microsomal
Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
<i>Arabidopsis thaliana</i>	130-158	LLVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:31)
<i>Glycine max</i>	126-154	LLVPYFSWKI SHRRHHSNTG SLDRDEVFV (SEQ ID NO:32)
<i>Zea mays</i>	136-164	LMVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:33)
<i>Ricinus communis</i> ^a	30- 58	LLVPYFSWKH SHRRHHSNTG SLERDEVFV (SEQ ID NO:34)
<i>Brassica napus D</i>	130-158 ^b	LLVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:31)
<i>Brassica napus F</i>	130-158 ^c	LLVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:31)

^a from plasmid pRF2-1C; ^bpositions 130-158 of SEQ ID NO:10; ^cpositions 130-158 of SEQ ID NO:14

Please amend Table 3 on page 13 as follows:

TABLE 3

Alignment of Amino Acid Sequences from Microsomal
Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
<i>Arabidopsis thaliana</i>	298-333	DRDYGILNKV FHNITDTHVA HHLFSTMPHY NAMEAT (SEQ ID NO:35)
<i>Glycine max</i>	294-329	DRDYGILNKV FHHITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:36)
<i>Zea mays</i>	305-340	DRDYGILNRV FHNITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:37)
<i>Ricinus communis</i> ^a	198-224	DRDYGILNKV FHNITDTQVA HHLF TMP (SEQ ID NO:38)
<i>Brassica napus D</i>	299-334 ^b	DRDYGILNKV FHNITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:66)
<i>Brassica napus F</i>	299-334 ^c	DRDYGILNKV FHNITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:66)

^a from plasmid pRF2-1C; ^bpositions 299-334 of SEQ ID NO:10; ^cpositions 299-334 of SEQ ID NO:14

Please amend Table 4 on page 14 as follows:

TABLE 4

Alignment of Conserved Amino Acids from Microsomal
Delta-12 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
<i>Arabidopsis thaliana</i>	165-180	IKWYGKYLNN PLGRIM (SEQ ID NO:39)
<i>Glycine max</i>	161-176	VAWFSLYLNN PLGRAV (SEQ ID NO:40)
<i>Zea mays</i>	172-187	PWYTPYVYNN PVGRVV (SEQ ID NO:41)
<i>Ricinus communis</i> ^a	65- 80	IRWYSKYLNN PPGRIM (SEQ ID NO:42)
<i>Brassica napus D</i>	165-180 ^b	IKWYGKYLNN PLGRTV (SEQ ID NO:67)
<i>Brassica napus F</i>	165-180 ^c	IKWYGKYLNN PLGRTV (SEQ ID NO:67)

^a from plasmid pRF2-1C; ^bpositions 165-180 of SEQ ID NO:10; ^cpositions 165-180 of SEQ ID NO:14

Please amend Table 5 on page 14 as follows:

TABLE 5

Alignment of Conserved Amino Acids from Plastid and Microsomal
Delta-15 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
<i>Arabidopsis thaliana</i> ^a	156-177	WALFVLGHD CGHGSFSNDP KLN (SEQ ID NO:43)
<i>Brassica napus</i> ^a	114-135	WALFVLGHD CGHGSFSNDP RLN (SEQ ID NO:44)
<i>Glycine max</i> ^a	164-185	WALFVLGHD CGHGSFSNNS KLN (SEQ ID NO:45)
<i>Arabidopsis thaliana</i>	94-115	WAIFVLGHD CGHGSFSDIP LLN (SEQ ID NO:46)
<i>Brassica napus</i>	87-109	WALFVLGHD CGHGSFSNDP RLN (SEQ ID NO:44)
<i>Glycine max</i>	93-114	WALFVLGHD CGHGSFSDSP PLN (SEQ ID NO:48)

^a Plastid sequences

Please amend Table 6 on page 14 as follows:

TABLE 6

Alignment of Conserved Amino Acids from Plastid and Microsomal
 Delta-15 Fatty Acid Desaturases

Species	Position	Amino Acid Sequence
<i>A. thaliana</i> ^a	188-216	ILVPYHGWRI SHRTHHQNHG HVENDESWH (SEQ ID NO:49)
<i>B. napus</i> ^a	146-174	ILVPYHGWRI SHRTHHQNHG HVENDESWH (SEQ ID NO:49)
<i>Glycine max</i> ^a	196-224	ILVPYHGWRI SHRTHQHGHG HAENDESWH (SEQ ID NO:51)
<i>A. thaliana</i>	126-154	ILVPYHGWRI SHRTHHQNHG HVENDESWV (SEQ ID NO:52)
<i>Brassica napus</i>	117-145	ILVPYHGWRI SHRTHHQNHG HVENDESWV (SEQ ID NO:52)
<i>Glycine max</i>	125-153	ILVPYHGWRI SHRTHHQNHG HIEKDESWV (SEQ ID NO:54)

^a Plastid sequences

Please amend the paragraph beginning at page 17, line 1, as follows:

The seeds of several different fatty acid lines have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia 20110-2209, and have the following accession numbers.

Please amend the paragraph beginning at page 43, line 14 as follows:

A single base transition from T to A at nucleotide 515 of the F gene was detected in Q508 compared to the Westar sequence. The mutation changes the codon at this position from CTC to CAC, resulting in the non-conservative substitution of a [[non-polar residue, leucine, for a polar residue, histidine]] polar residue, histidine, for a non-polar residue, leucine, in the resulting gene

product. No mutations were found in the F gene sequence of IMC 129 compared to the F gene sequence of Westar.

Please amend Table 20 on page 44 as follows:

TABLE 20

Alignment of Amino Acid Sequences
of Cloned Canola Membrane Bound-Desaturases

Desaturase Gene	Sequence ^a	Position
Canola-fad2-D(mutant)	AHKCGH (SEQ ID NO:68)	109-114 of SEQ ID NO:12
Canola-Fad2-D	AHECGH (SEQ ID NO:69)	109-114 of SEQ ID NO:10
Canola-Fad2-F	AHECGH (SEQ ID NO:69)	109-114 of SEQ ID NO:14
Canola-FadC	GHDCAH (SEQ ID NO:55)	170-175
Canola-fad3 (mutant)	GHKCGH (SEQ ID NO:56)	94-99
Canola-Fad3	GHDCGH (SEQ ID NO:57)	94-99
Canola-FadD	GHDCGH (SEQ ID NO:57)	125-130

(FadD = Plastid delta 15, Fad3 = Microsomal delta-15),
(FadC = Plastid delta-12, Fad2 = Microsomal delta-12)

^a One letter amino acid code; conservative substitutions are underlined; non-conservative substitutions are in bold.

Please amend the paragraph beginning at page 44, line 19 as follows:

Transcription *in vivo* was analyzed by RT-PCR analysis of stage II and stage III developing seeds and leaf tissue. The primers used to specifically amplify delta-12 desaturase F gene RNA from the indicated tissues were sense primer 5'-GGATATGATGATGGTGAAGA-3' (SEQ ID NO:19) and antisense primer 5'-TCTTTCACCATCATCATATCC-3' (SEQ ID NO:20). The primers used to specifically amplify delta-12 desaturase D gene RNA from the indicated tissues were sense primer 5'-GTTATGAAGCAAAGAAGAAAC-3' (SEQ ID NO:21)

and antisense primer 5'-GTTTCTTCTTTGCTTCATAAC-3' (SEQ ID NO:22). The results indicated that mRNA of both the D and F gene was expressed in seed and leaf tissues of IMC 129, Q508 and wild type Westar plants.

Please amend the paragraph beginning at page 46, line 26 as follows:

The second plasmid, pIMC205, was prepared by inserting a mutated Fad3 gene in sense orientation into a disarmed Ti vector. The mutant sequence contained mutations at nucleotides 411 and 413 of the microsomal Fad3 gene described in WO93/11245, thus changing the sequence for codon 96 from GAC to AAG. The amino acid at codon 96 of the gene product was thereby changed from aspartic acid to lysine. See Table 20. A bean (*Phaseolus vulgaris*) phaseolin (7S seed storage protein) promoter fragment of 495 base pairs, starting with 5'-TGGTCTTTTGGT-3' (SEQ ID NO:59), was placed 5' to the mutant Fad3 gene and a phaseolin termination sequence was placed 3' to the mutant Fad3 gene. The phaseolin sequence is described in Doyle et al., (1986) J. Biol. Chem. 261:9228-9238) and Slightom et al., (1983) Proc. Natl. Acad. Sci. USA 80:1897-1901.

Please amend the paragraph beginning at page 49, line 5 as follows:

The Fad2-D gene was amplified once using Elongase[®] (Gibco-BRL). PCR primers were: 5'-CAUCAUCAUCAUCTTCTTCGTAGGGTTCATCG-3' (SEQ ID NO:23) and 5'-CUACUACUACUATCATAGAAGAGAAAGGTTTCAG-3' (SEQ ID NO:24) for the 5' and 3' ends of the gene, respectively.

Please amend the paragraph beginning at page 49, line 10 as follows:

The Fad2-F gene was independently amplified 4 times, twice with Elongase[®] and twice with Taq polymerase (Boehringer Mannheim). The PCR primers used were: 5'-CAUCAUCAUCAUCATGGGTGCACGTGGAAGAA3' (SEQ ID NO:25) and 5'-CUACUACUACUATCTTTCACCATCATCATATCC3' (SEQ ID NO:26) for the 5' and 3' ends of the gene, respectively.